# REPORTS

## Effects of Alcohol Consumption on Plasma and Urinary Hormone Concentrations in Premenopausal Women

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***Background:*** **Most epidemiologic studies of the relationship between alcohol consumption and breast cancer risk over the past decade have shown that persons who consume a moderate amount of alcohol are at 40%-100% greater risk of breast cancer than those who do not consume alcohol. Dose–response effects have been observed, but no causal relationship has been established.** ***Purpose:*** **This study examines the hypothesis that alcohol consumption affects levels of reproductive hormones.** ***Methods:*** **A controlled-diet study lasting for six consecutive menstrual cycles was conducted. Participants were randomly assigned to two groups, and a crossover design was used. During the last three menstrual cycles, alcohol consumption of the two groups was reversed. Thirty-four premenopausal women, aged 21-40 years, with a history of regular menstrual cycles, consumed 30 g of ethanol (equivalent to approximately two average drinks) per day for three menstrual cycles and no alcohol for the other three. All food and alcohol consumed were provided by the study. Caloric intake was monitored to ensure that each woman would maintain body weight at approximately the baseline level. Hormone assays were performed on pooled plasma or 24-hour urine specimens collected during the follicular (days 5-7), peri-ovulatory (days 12-15), and mid-luteal (days 21-23) phases of the third menstrual cycle for subjects on each diet.** ***Results:*** **Alcohol consumption was associated with statistically significant increases in levels of several hormones. Plasma dehydroepiandrosterone sulfate levels were 7.0% higher in the follicular phase ($P = .05$). In the peri-ovulatory phase, there were increases of 21.2% ($P = .01$) in plasma estrone levels, 27.5% ($P = .01$) in plasma estradiol levels, and 31.9% ($P = .009$) in urinary estradiol levels. In the luteal phase, urinary estrone levels rose 15.2% ($P = .05$), estradiol levels increased 21.6% ($P = .02$), and estriol levels rose 29.1% ($P = .03$). No changes were found in the percent of bioavailable estradiol, defined by the sum of percent free estradiol and percent albumin-bound estradiol. However, increased total estradiol levels in the peri-ovulatory phase suggest elevated absolute amounts of bioavailable estradiol.** ***Conclusion:*** **This study has shown increases in total estrogen levels and amount of bioavailable estrogens in association with alcohol consumption in premenopausal women.** ***Implication:*** **This possible explanatory mechanism for a positive association between alcohol consumption and breast cancer risk merits further investigation. [J Natl Cancer Inst 85:722–727, 1993]**

Over the past decade, a number of case–control and cohort studies have examined the relationship between alcohol consumption and breast cancer (*1-35*). The majority of these studies indicate a positive association between alcohol consumption and breast cancer risk, with increased risks in the range of 40%-100% in moderate drinkers versus nondrinkers. Moreover, dose–response effects have been observed. However, a causal relationship has not been established, and mechanistic explanations for this association are speculative.

The literature on hormone levels and breast cancer risk is controversial (*36*). However, a large number of breast cancer risk factors appear to have hormonal components, suggesting an etiologic role for endogenous hormones. In this study, we investigated the effect of alcohol consumption on levels of endogenous reproductive hormones.

## Subjects and Methods

### Participants

Participants were recruited by posters and newspaper advertisements from communities around Beltsville, Md. Volunteers were screened to eliminate women who currently smoked, had health problems (such as cancer or diabetes), used oral contraceptives in the past year, regularly used medications, reported menstrual irregularities, were pregnant or had breast-fed in the past year, or had dietary patterns (such as vegetarian diets or extensive use of supplements) that might make compliance with the study diet more difficult. Women weighing less than 80% or greater than 130% of the levels given in the 1983 Metropolitan Life Insurance Table (*37*) were excluded. Thirty-seven women, aged 21-40 years, completed the study. Three women were eliminated from these analyses because of menstrual irregularities. Thirty women reported educational levels; all but one completed high school, and 17 had completed college. The study was approved by the institutional review boards of the National Cancer Institute and of Georgetown University School of Medicine.[1]

### Experimental Design

Women were randomly assigned to one of two diet groups by use of a table of random numbers. Resulting groups were checked to be sure they

*See "Notes" section following "References."

were balanced with respect to body mass index. All women consumed the same diet, on a 14-day repeating menu. All food and beverages were provided by the study. During the first three menstrual cycles, one group consumed approximately 30 g of alcohol (equivalent to about two average drinks) per day, while the other group consumed no alcohol. During the last three menstrual cycles, alcohol consumption of the two groups was reversed. Menus provided 1400-2800 kcal in 200-kcal increments. To maintain body weight, each woman was weighed daily, and whenever she gained or lost at least 1 kg and maintained this change for at least 3 days, she was moved from one caloric level to another. The women consumed no vitamin or mineral supplements during the study. The only alcohol that they consumed was that provided by the study. Alcohol was consumed shortly before bedtime and was in the form of 95% ethanol in fruit juice. Activities were restricted after consumption. Calories derived from alcohol were compensated for by a cola drink during non–alcohol-consumption cycles. Women were encouraged to report any deviation from the study diet. All meals were prepared in the Beltsville Human Nutrition Research Center. On weekdays, morning and evening meals were eaten in the Center's dining facility and a carry-out lunch was provided. Weekend meals were packaged for home consumption. Diets provided 35%-37% calories from fat, a P : S : M ratio (ratio of fat as percent calories from polyunsaturates : saturates : monounsaturates) of 0.6 : 1.0 : 1.0, 8.3 g of fiber per 1000 kcal, and 150 mg of cholesterol per 1000 kcal.

## Hormone Assays

Blood specimens of 10 mL were taken from the women after fasting and were analyzed for hormone levels. The specimens were drawn between 6 and 8 AM on days 5-7, 12-15, and 21-23 of the third and sixth menstrual cycles. Equal volumes of daily plasma were pooled from days 5-7 (indicated by F), days 12-15 (indicated by O), and days 21-23 (indicated by L) of the menstrual cycle. Ideally, these pools correspond to early follicular (F), peri-ovulatory (O), and mid-luteal (L) phases of the menstrual cycle and will be so indicated. Twenty-four-hour urine collections were obtained for the same days as the plasma specimens, and corresponding pools were formed. The following hormone analyses were performed on each of the three pools (F, O, and L): (*a*) in the plasma—measurement of dehydroepiandrosterone sulfate, androstenedione, estrone sulfate, estrone, estradiol, testosterone, and sex hormone-binding globulin levels as well as percent albumin-bound estradiol and percent unbound estradiol; and (*b*) in the urine—measurement of levels of estrone, estradiol, estriol, 2-hydroxyestrone, and 2-hydroxyestradiol. Plasma prolactin was assayed on days 5-7 only, and plasma progesterone was assayed on days 21-23 only.

Dehydroepiandrosterone sulfate levels were measured by a radioimmunoassay kit from ICN-Biomedical (Costa Mesa, Calif.). Plasma levels of estrone, estradiol, testosterone, androstenedione, and estrone sulfate were measured by radioimmunoassay (*38,39*). The percent unbound estradiol and the percent albumin-bound estradiol were measured by centrifugal ultrafiltration (*40,41*). Sex hormone-binding globulin levels were measured by an immunoradiometric assay kit (Farmos Group Ltd., Oulunsalo, Finland). Inter-assay coefficients of variation were all less than 12%, and intra-assay coefficients of variation were all less than 10%.

Urine for analysis of estrone, estradiol, and estriol levels was hydrolyzed with β-glucuronidase, and the resultant unconjugated steroids were extracted with an organic solvent, chromatographed with LH-Sephadex (Pharmacia LKB, Piscataway, N.J.), and measured by radioimmunoassay. The levels of the catechol estrogens (2-hydroxyestrone and 2-hydroxyestradiol) were measured by radioimmunoassay with the use of antibody provided by Dr. S. C. Chattoraj (Boston University, Boston, Mass.) and purification procedure as described (*42*). Inter-assay coefficients of variation were 15% for 2-hydroxyestrone and 2-hydroxyestradiol, and intra-assay coefficients of variation were less than 11%.

Prolactin levels were measured by a radioimmunoassay kit from Ciba-Corning (East Walpole, Mass.), and progesterone levels were measured by a radioimmunoassay kit from ICN-Biomedical. Inter-assay and intra-assay coefficients of variation were less than 10% for both assays.

## Statistical Analyses

Statistical analyses were performed with the SAS Statistical Analysis System (*43*). Results obtained using logarithm transformations of hormone values were very similar to those for untransformed variables, and only data for untransformed variables are given. The study was designed to include a total of 44 hormone variables. Possible statistical effects of multiple comparisons should be kept in mind. Subjects responded to extensive questionnaires on such topics as demographics, health history, and alcohol-consumption history. Spearman correlation coefficients between all hormone variables and the following covariates were examined: self-reported usual number of drinks per week, height, weight, body mass index, age, lactation history, time since last lactation, number of pregnancies, number of live births, months since last pregnancy, smoking history, use of oral contraceptives, and age at menarche. No statistically significant differences between the two diet groups were observed for these covariates with the exception of history of ever having smoked ($P = .05$). This difference does not appear to be associated with the relationship of alcohol consumption and hormone levels. The effects of diet order on hormone values were determined by comparing group means of paired differences in hormone values. With the exception of plasma estrone on days 5-7 ($P<.01$) and percent albumin-bound estradiol on days 5-7 ($P = .05$), no statistically significant diet-order effects were observed. Hormone values in subjects on diets with and without alcohol were compared by Student's *t* tests for paired data.

# Results

Table 1 shows the characteristics of the study population with regard to a number of variables that might be related to hormone levels and/or breast cancer risk.

Spearman correlation coefficients between hormone values obtained in the women while on a diet containing no alcohol and values of several covariates were examined. A statistically significant correlation was found between dehydroepiandrosterone sulfate in the follicular ($r = .43$; $P = .01$) and peri-ovulatory ($r = .35$; $P = .04$) phases of the menstrual cycle and self-reported usual number of drinks consumed per week. Self-reported usual alcohol consumption also showed a statistically significant correlation with testosterone levels in the peri-ovulatory ($r = .43$; $P = .01$) and luteal ($r = .44$; $P = .01$) phases. The associated group of covariates, including ever pregnant, number of pregnancies, ever breast-fed, and number of live births, showed a statistically significant negative correlation with follicular-phase prolactin levels ($r = -.45$; $P = .01$) and percentage of total estradiol bound to albumin ($r = -.42$; $P = .02$).

Values for plasma and urinary hormones were determined during the third menstrual cycle of the women on diets with and without alcohol. Table 2 shows group means for these values for days 5-7, days 12-15, and days 21-23 of the menstrual cycle. The major result of this study indicates that

**Table 1.** Baseline characteristics of study population

| Variable | Measure |
|---|---|
| | ***Mean ± SD*** |
| Age, y | 30.4 ± 4.7 |
| Weight, kg | 64.8 ± 12.5 |
| Height, cm | 163.2 ± 6.6 |
| BMI, kg/m$^2$ | 24.4 ± 4.6 |
| Reported drinks per week | 1.7 ± 1.4 |
| Age at menarche, y | 12.6 ± 1.4 |
| | ***No. of subjects*** |
| ≥1 pregnancy* | 18 |
| ≥1 live birth | 16 |
| Ever breast-fed | 11 |
| Ever used oral contraceptives | 27 |
| Ever smoked | 13 |

*Average number of pregnancies = 2.2.

**Table 2.** Plasma and urinary hormone levels in subjects on controlled diet with or without alcohol

| Hormone, unit of measure* | Levels on days of menstrual cycle† | | | | | |
|---|---|---|---|---|---|---|
| | Days 5-7 (F) | | Days 12-15 (O) | | Days 21-23 (L) | |
| | With alcohol | Without alcohol | With alcohol | Without alcohol | With alcohol | Without alcohol |
| Plasma | | | | | | |
| DHEA-$SO_4$, μmol/L | 7.00 (2.93)‡ | 6.54 (2.55)‡ | 7.11 (2.52) | 6.81 (3.07) | 7.02 (3.07) | 6.54 (2.61) |
| $A^4$DIONE, nmol/L | 0.65 (0.20) | 0.61 (0.22) | 0.68 (0.24) | 0.69 (0.22) | 0.67 (0.22) | 0.66 (0.22) |
| $E_1SO_4$, nmol/L | 1.32 (0.74) | 1.25 (0.49) | 2.99 (2.47) | 2.66 (1.96) | 2.61 (1.83) | 2.29 (1.19) |
| Estrone, pmol/L | 159 (72) | 174 (71) | 297 (143)§ | 245 (114)§ | 253 (113) | 238 (105) |
| Estradiol, pmol/L | 140 (44) | 156 (75) | 306 (167)§ | 240 (137)§ | 249 (111) | 259 (126) |
| Progesterone, nmol/L | | | | | 37.2 (19.5) | 35.0 (18.4) |
| Prolactin, μg/L | 18.9 (8.4) | 18.1 (9.7) | | | | |
| Testosterone, nmol/L | 0.83 (0.59) | 0.80 (0.52) | 1.11 (0.69) | 1.04 (0.59) | 0.94 (0.55) | 0.90 (0.55) |
| Unbound estradiol, % | 2.20 (0.35) | 2.19 (0.33) | 2.25 (0.36) | 2.25 (0.31) | 2.19 (0.39) | 2.27 (0.39) |
| Estradiol bound to albumin, % | 34.5 (11.4) | 32.6 (10.4) | 35.3 (11.6) | 35.9 (12.1) | 33.0 (10.2) | 36.0 (10.4) |
| SHBG, nmol/L | 13.5 (11.1) | 14.0 (13.3) | 16.8 (12.0) | 16.9 (9.3) | 16.1 (11.0) | 13.4 (11.7) |
| Urine | | | | | | |
| Estrone, nmol/d | 18.6 (9.3) | 20.0 (13.0) | 35.7 (18.6) | 31.7 (13.2) | 31.1 (14.0)‡ | 27.0 (14.8)‡ |
| Estradiol, nmol/d | 10.2 (4.6) | 9.1 (3.9) | 18.6 (11.8)§ | 14.1 (6.8)§ | 15.2 (8.2)‡ | 12.5 (6.3)‡ |
| Estriol, nmol/d | 14.1 (9.5) | 15.6 (8.5) | 29.4 (21.7) | 27.9 (20.5) | 35.9 (26.9)‡ | 27.8 (20.3)‡ |
| 2-OH-$E_1$, nmol/d | 23.0 (17.8) | 23.8 (20.3) | 23.0 (18.7) | 24.6 (21.7) | 24.7 (21.6) | 25.2 (19.9) |
| 2-OH-$E_2$, nmol/d | 10.9 (5.8) | 24.4 (21.5) | 12.3 (9.3) | 13.6 (11.4) | 11.6 (7.5) | 11.3 (7.5) |

*DHEA-$SO_4$ = dehydroepiandrosterone sulfate; $A^4$DIONE = androstenedione; $E_1SO_4$ = estrone sulfate; SHBG = sex hormone-binding globulin; 2-OH-$E_1$ = 2-hydroxyestrone; 2-OH-$E_2$ = 2-hydroxyestradiol.

†Equal volumes of daily plasma were pooled from days 5-7 (indicated by F), days 12-15 (indicated by O), or days 21-23 (indicated by L) of the menstrual cycle. Values in columns = means (SD).

‡$.01 < P \leq .05$.

§$P \leq .01$.

alcohol consumption was associated with statistically significant increases (as determined by Student's $t$ test for paired data) in the levels of several hormones. Plasma dehydroepiandrosterone sulfate levels increased in the follicular phase by 7.0% ($P$ = .05). An increase of 7.3% ($P$ = .06) was also observed in the level of this hormone in the luteal phase. In the peri-ovulatory phase of the menstrual cycle, plasma estrone levels increased by 21.2% ($P$ = .01) and plasma estradiol levels increased by 27.5% ($P$ = .01). This increase was accompanied by a 31.9% ($P$ = .009) increase in the levels of urinary estradiol. These increases in hormone levels in the peri-ovulatory phase were followed in the luteal phase by increases in the levels of urinary estrone (15.2%; $P$ = .05), estradiol (21.6%; $P$ = .02), and estriol (29.1%; $P$ = .03). The levels of the catechol estrogens 2-hydroxyestrone and 2-hydroxyestradiol did not show statistically significant differences during the cycle or between the consumption of the alcohol and non-alcohol diets. The standard deviations were large, however, which may have obscured minor changes. None of the hormones studied showed a statistically significant decrease in levels with alcohol consumption.

Examination of the progesterone values for days 21-23 of the menstrual cycles revealed eight cycles (of a total of 68) with levels less than 3 ng/mL. In seven of these cycles, the progesterone levels were less than 1 ng/mL. In two cases, a luteinizing hormone peak was not identified, making anovulation likely. When these two women were eliminated from the hormone analyses, the results were essentially the same as those reported above. In three additional cycles, urinary luteinizing hormone levels peaked at days 18, 19, and 21 of the menstrual cycle. In these cycles, the progesterone levels may not yet have risen. In the remaining three cycles, luteinizing hormone peaks have been identified, which offer no explanation for the progesterone results. Analyses were performed eliminating all eight women who had a cycle with a low progesterone level. This resulted in a sample size of 26 instead of 34, with accompanying reduction in power. In this analysis, with the reduced sample size, statistically significant increases ($P$ = .05) were again observed for plasma estradiol levels (21.4%; $P$ = .05) and urinary estradiol levels (24.7%; $P$ = .05) at days 12-15 and for urinary estradiol levels (19.3%; $P$ = .05) at days 21-23. The increase in dehydroepiandrosterone sulfate levels at days 5-7 retained approximately the same magnitude (7.2%) while decreasing slightly in statistical significance ($P$ = .06). Increases in plasma estrone levels at days 12-15 (15.0%; $P$ = .11) and urinary estrone levels (12.6%; $P$ = .17) and estradiol levels (21.4%; $P$ = .12) at days 21-23 of the cycle decreased in magnitude and did not remain statistically significant.

For each of the seven variables which increased significantly in association with alcohol consumption in the 34 subjects, we determined the percentage of women whose hormone levels increased. This percentage ranged from a low of 59% for peri-ovulatory plasma estradiol levels to a high of 71% for luteal urinary estriol levels and averaged 65%. All of the women studied showed an increase in the levels of at least one of these hormones, and 71% of the women showed an increase in the levels of four or more of the hormones.

## Discussion

To our knowledge, this is the first controlled-diet study to examine the association between alcohol consumption and hormone levels. Previous studies examining hormonal effects of alcohol consumption were largely limited to studies in alcoholics, in whom it is difficult to distinguish specific effects of alcohol from generalized liver damage (*44-49*), and studies involving administration of acute, large doses of alcohol to non-alcoholic premenopausal or postmenopausal women followed by short periods of observation and sampling of blood and/or urine (*50-54*). Two recent cross-sectional studies (*55,56*) of alcohol consumption involved postmenopausal and perimenopausal women, respectively. They showed no statistically significant association between alcohol consumption and estradiol or estrone levels. These studies differed from our study in being observational, involving older women, and having reported alcohol intakes generally lower than those we provided. Thus, there is little information available on the effects of alcohol, consumed over fairly long periods, on non-alcoholic, premenopausal women.

Since diet order did not have a statistically significant influence on hormone values, the effects observed here appear to be reversible if sufficient time is allowed. However, the effects of drinking patterns over many years may or may not be reversible. To this point, we observed that levels of dehydroepiandrosterone sulfate during nondrinking cycles were significantly correlated with reported frequency of alcohol consumption. This observation is compatible with an effect of alcohol on adrenal steroidogenesis, since the source of dehydroepiandrosterone sulfate is the adrenal gland (*57*). A recent cross-sectional study (*55*) observed an association between moderate alcohol consumption and cortisol levels, also an adrenal effect.

Other investigators (*58-60*) have also observed the negative correlation of the group of covariates, including pregnancy, childbirth, and breast-feeding, with prolactin levels. Effects of first birth on reproductive hormone levels is somewhat controversial (*61-64*). Levels of sex hormone-binding globulin observed in this study were significantly lower than those observed by others (*65-67*). This may affect the ability to detect differences in sex hormone-binding globulin associated with alcohol consumption.

One very important strength of this study was the examination of three phases of the menstrual cycle. This study design allowed patterns of change in hormone levels over the menstrual cycle to be observed. Increases in plasma estrone and estradiol levels in the peri-ovulatory phase of the menstrual cycle are followed by increases in urinary estrone, estradiol, and estriol levels in the luteal phase. Even though luteal and peri-ovulatory pools were temporally separated by several days, it is likely that metabolism of increased amounts of estrone and estradiol in the plasma is responsible for luteal increases in the levels of urinary hormones. These patterns strengthen the conclusions of the study. In addition to patterns over the menstrual cycle, simultaneous changes in estrone and estradiol levels in the plasma and in estrone, estradiol, and estriol levels in the urine point to major effects of alcohol on estrogen production and, perhaps, metabolism. Major changes in metabolic pathways are unlikely, however, since no difference in catechol estrogen excretion was observed between the diets.

No effects on percentage of bioavailable estradiol, defined by the sum of percent free estradiol and percent albumin-bound estradiol, were observed. However, increases in plasma estrone and estradiol levels translate into greater absolute amounts of bioavailable estradiol.

There were several occurrences of low progesterone levels during days 21-23 that are not consistent with a true luteal phase. In three cases, these low progesterone levels during days 21-23 were associated with late luteinizing hormone peaks, also bringing into question the truly "peri-ovulatory" nature of the pool from days 12-15 for these subjects. The results of analyses omitting relevant subjects are given. Even the analysis omitting all subjects in whom the peri-ovulatory and/or luteal nature of the pools can be questioned shows significantly increased plasma and urinary estradiol levels during days 12-15 (peri-ovulatory phase) and significantly increased urinary estradiol levels during days 21-23 (luteal phase). These menstrual-cycle issues point to the need for further and possibly larger studies to confirm and refine the results reported here.

In summary, this study has shown increases in total estrogen levels and amount of bioavailable estrogens in association with alcohol consumption in premenopausal women. This possible explanatory mechanism for a positive association between alcohol consumption and breast cancer risk merits further investigation.

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## Notes

[1]The U.S. Department of Agriculture has a cooperative research agreement with Georgetown University to support human studies.

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We thank Lisa L. Kahle, B.A., for management of databases and statistical programming; Matthew Sunkin, B.S., for participation in study recruitment and coordination; and Priscilla Stelle, R.D., and Evelyn Lashley, R.D., for management of the dietary facility.

Manuscript received September 22, 1992; revised January 19, 1993; accepted January 26, 1993.

# Nm23 Protein Expression in Ductal In Situ and Invasive Human Breast Carcinoma

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***Background:*** **Mortality associated with human breast carcinoma is almost entirely due to subsequent metastatic disease, but the molecular basis of this metastasis is not understood. Elucidation of the genetic control of metastatic propensity of a tumor is important in determining prognosis and choice of therapy. Expression of nm23, a putative metastasis suppressor gene, has been detected in human breast cancers, but studies have not consistently shown high levels of the Nm23 messenger RNA or protein to be associated with better histological differentiation. This inconsistency suggests that Nm23 protein may act independently as a metastasis suppressor.** ***Purpose:*** **The purpose of this retrospective study was to investigate the relationship of Nm23 protein expression with 1) histology in ductal breast carcinoma in situ and 2) the variables considered to be the major prognostic indicators in invasive breast carcinoma.** ***Methods:*** **We obtained formalin-fixed biopsy specimens of breast tissue excised from 128 patients with breast lesions detected by mammography. Of these patients, 35 had been diagnosed with benign breast disease, 26 with ductal carcinoma in situ (DCIS), and 67 with invasive carcinoma. Tissue sections were embedded in paraffin blocks, and immunohistochemical staining was used to determine Nm23 expression. Specimens were rated positive if all lesional epithelium was stained and negative if any lesional epithelium was unstained. Statistical analysis was performed by multiple regression analysis because of nonorthogonality of the data.** ***Results:*** **All 35 examples of benign breast disease showed uniform epithelial cell staining. The seven cases of comedo DCIS were negative for Nm23 protein; all 18 noncomedo types were positive. Nm23 negativity was significantly associated with worsening invasive ductal carcinoma grade and advancing lymph node stage but not with tumor diameter or vascular invasion. Despite the putative antimetastatic role of the nm23 gene, no statistically significant association was found between Nm23 protein expression and vascular invasion.** ***Conclusions:*** **The precise role of the nm23 gene remains to be established, but our simplified immunohistochemical rating system shows an association between Nm23 protein expression and the two most significant prognostic factors relating to histologic grade and stage. Nm23 negativity distinguished comedo ductal carcinoma in situ from the other histological types, a finding consistent with the fact that comedo histology is known to have a higher likelihood of becoming invasive and of having higher cell proliferation rates and higher expression of growth factor (c-erb B2) receptor. [J Natl Cancer Inst 85:727–731, 1993]**

The mortality associated with human breast carcinoma is almost entirely caused by subsequent metastatic disease; however, the molecular basis underlying this process is ill-defined, and elucidating the genetic control of the metastatic propensity of a tumor is important in determining prognosis and choice of therapy. Patient prognosis in breast carcinoma is largely based on the disease stage [i.e., the size of the primary tumor, involvement of regional lymph nodes, and distant spread (*1*)]; grade (*2*); histologic type (*3*); features of aneuploidy and proportion of cells undergoing mitosis; and the presence of estrogen and progesterone receptors, which currently act as independent prognostic indicators (*1,4,5*).

The cloning and identification of nm23 as a putative "metastasis suppressor" gene have generated considerable interest. The nm23-H1 gene is located on chromosome 17q (q1.1 → q2.1) (*6*) in a region distinct from the tumor suppressor gene p53, which is located on 17p13. The gene was first isolated by differential hybridization analysis of murine K1735 melanoma cell lines of high and low metastatic ability (*7*); further studies in a number of models demonstrated an inverse correlation between nm23 gene expression and metastasis (*7-9*). Additional evidence implicating nm23 in the control of metastatic ability has come from studies in mice, where highly metastatic K1735 TK murine melanoma cells transfected with nm23 demonstrated a significantly reduced metastatic potential independent of tumor cell proliferation (*10*). The nm23-H1 gene is highly conserved, and its product shows complete identity to that of human erythrocyte nucleoside diphosphate (NDP) kinase (*11,12*). A related human gene, named nm23-H2, shows 88% homology with nm23-H1, although its significance as a "metastasis suppressor gene" has not yet been determined (*13*).

Several reports have now associated a reduced expression of nm23 gene activity with a poor prognosis in human breast carcinoma. In a preliminary report by Bevilacqua et al. (*14*) and a large study by Hennessy et al. (*15*), a reduction of nm23 messenger RNA in biopsy tissue was positively correlated with the nodal status of individuals and negatively with the disease-free survival of the patients.

*See "Notes" section following "References."